PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43*bis*.1)

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

Applicant's or agent's file reference
see form PCT/ISA/220

FOR FURTHER ACTION
See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/006698	21.06.2004	20.06.2003

International Patent Classification (IPC) or both national classification and IPC
C12N5/06, C12N5/10, A61K35/34, C12Q1/02, A61P9/04

Applicant
AXIOGENESIS AG

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the international application
- ☒ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/006698

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 76-79

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 76-79

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes: Claims	2-8, 11, 13, 14, 21-44
	No: Claims	1, 9, 10, 12, 15-20, 45-58, 80, 81
Inventive step (IS)	Yes: Claims	21-39, 43, 44
	No: Claims	1-20, 40, 42, 45-75, 80, 81
Industrial applicability (IA)	Yes: Claims	1-16, 21-49, 57-75, 80, 81
	No: Claims	-

2. Citations and explanations

see separate sheet

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 17-20 and 50-56 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

No opinion will be formulated with respect to the reach-through claims 76-79 and those parts of claims 80 and 81 which refer to claims 76-79 because no International Search Report has been established for these claims (Rule 6.2(a)(vi) PCT).

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1 WO 03/010303 A 6 February 2003

D1a Mummery C. *et al.*, *Circulation* 6 June 2003, 107(21), 2733-2740 (online 12 May 2003)

D2 WO 02/051987 A 4 July 2002

D3 US 5,733,727 A 31 March 1998

D4 Müller M. *et al.*, *FASEB Journal* December 2000, 14(15), 2540-2548

D5 Spielmann H. *et al.*, *Congenital Anomalies* December 2000, 40(Suppl.), S8-S18

D6 Kettenhofen R. *et al.*, *Naunyn-Schmiedeberg's Archives of Pharmacology* March 2002, 361 (Suppl. 1), R154, abstract 601

D7 Kolossov E. *et al.*, *Tissue Engineering* August 2003, 9(4), pages 853-854, abstract 230 Second Meeting of the European Tissue Engineering Society; Genoa, Italy, 3-6 Sept. 2003

Examples 1, 4 and 5 of D1 and the corresponding article D1a teach the differentiation of embryonic stem cells (ES) by co-culture with visceral endoderm END-2 cells; examples 2 and 3 of D1 further describe the differentiation of ES into skeletal muscle cells and vascular endothelial cells by co-culturing ES with ectoderm or endoderm derived cells. In view of D1 and D1a, the subject-matter of claims 1, 9, 10, 12, 15-17, 45-48, 50, 80 and 81 (cells, tissues) is not new (Article 33(2) PCT) and the further subject-matter of claims 2-8, 11, 13, 14, 40-42, 49 and 62-75 does not involve an inventive step (Article 33(3) PCT). This Authority observes that the wording of the claims does not restrict the origin or the level of differentiation of the embryonic cells of the second cell type.

D2 (examples) teaches the transfection of ES with resistance and reporter genes under the control of

a cardiac specific promoter and the selection of differentiated cardiomyocytes; applications of the cells in screening and therapy are foreseen (p. 37). In view of D2, the subject-matter of claims 18-20, 45-58, 80 and 81 (cells, tissues, vectors) is not new (Article 33(2) PCT) and the subject-matter of claims 59-61 does not involve an inventive step (Article 33(3) PCT). This Authority observes that the wording "cardiomyocytes, fibroblasts and/or endothelial cells" in claims 51-53 includes the case where only cardiomyocytes are present.

D3 (example 4) and D4 (see "Materials and Methods", p. 2541-2542) teach the differentiation of ES cells into cardiomyocytes and the selection of cardiomyocytes using a reporter gene under the control of a cardiac specific promoter; therapeutic applications are foreseen (D3, examples 1-3 and 5; D4, last paragraph, p. 2547), as well as screening assays (D4, last paragraph, p. 2547). In view of D3 and D4, the subject-matter of claims 18-20, 45-58, 62-75, 80 and 81 (cells, tissues, vectors) is not new (Article 33(2) PCT) and the subject-matter of claims 59-61 does not involve an inventive step (Article 33(3) PCT).

D5 and D6 teach the use of ES transfected with a reporter gene under the control of a cardiac specific promoter and of cardiomyocytes derived from these ES in toxicity tests *in vitro*. In view of D5 and D6, the subject-matter of claims 45-49, 57, 58, 62-75, 80 and 81 is not new (Article 33(2) PCT) and the subject-matter of claims 59-61 does not involve an inventive step (Article 33(3) PCT).

The problem underlying the application is the provision of an alternative process for the derivation of cardiomyocytes and cardiac tissue from ES; the solution provided is a parallel selection process where ES cells are transfected with selectable markers under the control of promoters specific for cardiomyocytes and for fibroblasts and/or endothelial cells, allowing for the selection among the differentiated cells of the desired cardiomyocytes together with cells supporting said cardiomyocytes. The closest prior art is represented equally by D1/D1a, which disclose the co-culture of ES with embryonic endothelial cells to support differentiation to cardiomyocytes, and by D2, which discloses a selection strategy for cardiomyocytes alone using selectable markers similar to those of the invention. None of the prior art documents suggests to derive simultaneously several cell types from ES and to allow these cell types to support each other and to give rise to cardiac tissue. The process of claims 21-39 is thus new and an inventive step can be acknowledged.

The products of claims 43 and 44 can be distinguished from similar products of the prior art, such as those described in D1/D1a and D2, in that, regardless of their type, all the cells comprise selectable markers under the control of at least two regulatory sequences specific for different cell types. While these selectable marker constructs were known in the art (D2), and their presence does not provide the differentiated cells with any further property which would be useful with respect to therapy or screening assays, the prior art does not provide any incentive to transfect cells of one lineage with selection marker constructs for another lineage, as would be required to obtain products identical to those of claims 43 and 44. Therefore, the products of claims 43 and 44 meet the requirements of novelty and inventive step regardless of the process in which they are obtained.

The subject-matter of claims 21-39, 43 and 44 thus meets the requirements of Article 33 PCT.

For the assessment of the present claims 17-20 and 50-56 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims.

Re Item VI
Certain documents cited

D7 does not belong to the state of the art under Rule 64.1 PCT as the priority date of 20 June 2003 can be allowed for subject-matter derivable from the present examples 1-5.

Re Item VII
Certain defects in the international application (form, content)

With all due respect to Pr. McLuhan, this Authority questions the relevance of his observations about the media and their impact on the culture environment (Cf. p. 43, lines 6-7) for the understanding of the present application. For instance, the media do not appear to be able to massage the cardiac tissues of the examples.

Re Item VIII
Certain observations on the international application (clarity, support)

The present set of claims contravenes Article 6 PCT.

Claims 15 and 16 pertain to cells and tissues of claims 1-14, while said claims 1-14 actually pertain to a method of modelling tissues; claim 57 pertain to vectors of claims 51-56, and claim 58 refers to those vectors, while said claims 51-56 actually pertain to a method for improving cardiac function in a mammal; in all cases, the reader has to construe the technical features of the claimed products (cells, tissues, vectors) from the technical features of methods by which the products are produced (claims 1-14) or in which the products are used (claims 51-56) although the features of said methods impart properties onto the products only to a limited extend and, as a result, the matter for which protection is sought is not clearly defined (Article 6 PCT).

Claims 40-50, 59, 60, 62-75, 80 and 81 make use of dependency from multiple independent claims or refer to multiple independent claims, and of those, multiple dependent claims 45-50, 59, 60, 62-75, 80 and 81 make reference to multiple dependent claims in contravention of Rule 6.4 PCT.

Claim 61 does not meet the requirements of Articles 5 and 6 PCT in that the claimed apparatus is neither disclosed nor supported by the description: The description provides no actual guidance as to the technical features of an apparatus specially adapted for analysing the array of claim 59 which would allow a skilled person to build such an apparatus.

This Authority regards the number of claims, eighty-one in total of which no less than twenty-four

are independent claims, as excessive and unreasonable in regard of the nature of the invention (Rule 6.1(a) PCT).

Claims 1-17 and 21-50 are drawn to any tissue or organ, while the supplied examples only teach the modelling of cardiac tissue. The skilled person wanting to derive, according to the method of the invention, a tissue other than cardiac tissue, such as pancreatic tissue or neural tissue, would find in the application neither disclosure nor guidance as to the suitable cell types to select as the second or further cell types to support the first cell type which characterise the target tissue, and it is not even clear what the benefits of the method of tissue modelling of the invention would be when applied to tissues other than cardiac tissue. Given the poorly predictable nature of biology, extending the teachings of the present application to further tissues is likely to involve extensive experimentation, and may well require the use of inventive skill. In the absence of at least one further representative example with a different tissue, this Authority regards the subject-matter pertaining to tissues other than cardiac tissue as lacking proper support and disclosure in the application (Article 5; PCT Guidelines 5.45-5.51).